

## The use of the brine shrimp *Artemia* in aquaculture

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### Introduction

One of the major differences between aquaculture and cattle breeding is that the larvae of most aquatic animal species of commercial interest, which are grown in intensive hatchery systems, have to be offered a live food whereas cattle accept inert diets throughout their live cycle (Kinne and Rosenthal, 1977).

Culturing of the zooplankton that normally constitutes the natural food of fish and shrimp larvae, is either commercially unfeasible or technically hard to realize (Girin and Person-Le Ruyet, 1977). As a result "... the efforts of early pioneers to rear marine fish were hampered by inadequate and unsuitable larval food supplies" (Shelbourne, 1968).

A very significant progress in hatchery aquaculture was made with the discovery by Seale (1933) in the USA and Rollesfsen (1939) in Norway, that the 0.4 mm nauplius larva of *Artemia* constitutes an excellent food source for newborn fish larvae.

<sup>1</sup> "Bevoegdverklaard Navorser" at the Belgian National Foundation for Scientific Research (NFWO).

Technically speaking the advantage of using *Artemia* is that one starts from an apparently inert product, namely the dry cysts. These cysts which are in fact inactive embryos are commercially available, can be stored for years and only have to be incubated for 24 hr in seawater to produce free-swimming larvae. Furthermore, brine shrimp are very well accepted as a food source. It is not exactly known if this is due to their biochemical composition, their very thin carapace, the fact that they are a moving prey (swimming) or a combination of all these factors.

*Artemia* has been found to be a suitable food for the most diversified groups of organisms of the animal kingdom, e.g. foraminifers, coelenterates, flatworms, polychaetes, squids, insects, chaetognaths, and of course a wide variety of both marine and freshwater crustaceans and fishes. In his treatise on "Cultivation of marine organisms", Kinne (1977) pertinently indicated that "... more than 85% of the marine animals cultivated thus far have been offered *Artemia salina* as food source, either together with other foods or, more often, as a sole diet".

It has been proved many times that a diet of live *Artemia* gives better results than any preparation of dead brine shrimp (Serfling *et al.*, 1974; Carlberg and Van Olst, 1975; Beck, 1979; Schauer *et al.*, 1979). The recent finding by Flüchter (1980) that "... whitefish larvae get through metamorphosis equally well whether they are given *Artemia* that is shock-frozen in liquid nitrogen (-196 °C) or living *Artemia*", but not when given slow-frozen nauplii, point to the fact that essential substance(s) is (are) lost during freezing and freeze-drying. In a few cases, it has been demonstrated that dried brine shrimp can be successfully used as protein source in pelletized diets for fish and shrimp (Deshimaru and Shigueno, 1972; Gabaudan *et al.*, 1980).

In most cases brine shrimp are used as freshly-hatched nauplii. Although outgrown *Artemia* larvae are reported to be a better food than nauplii for many predators (Kelly *et al.*, 1977; Purdom and Preston, 1977), the fact that they have to be cultured for a few days has limited this type of application in many aquaculture hatcheries (Brouillet, 1977). Adult brine shrimp are harvested from saline biotopes as food for the larvae of lobsters (Shleser and Gallagher, 1974) and the freshwater prawn *Macrobrachium rosenbergii* (Anonymous, 1978).

### Historical aspects of the "supply and demand" of cysts

Initially the commercial supply of cysts, first from saltponds in the San Francisco Bay area (California, USA) and later also from Great Salt Lake (Utah, USA) and Little Manitou Lake (Saskatchewan, Canada), seemed to be unlimited. The exponentially increasing demand of brine shrimp cysts by aquarium hobbyists and aquaculture hatcheries, however, soon exceeded by far the yearly harvest of approximately 30 to 50 metric tons. From the late sixties on, the dramatic impact of the aggravating cyst shortage on the expansion of aquaculture was repeatedly underlined at international conferences (Provasoli, 1969; FAO, 1972, 1976; ASEAN, 1976, 1977). Resolutions, such as the one taken by FAO that "... a fuller exploration and exploitation of the world's resources of *Artemia* for aquaculture purposes were considered to be of special importance", all pointed to the urgency of the problem. The situation did not improve however; prices continued to soar, and the hatching quality of the product delivered became less and less reliable. When one was lucky enough to receive a brand of good quality, only 4 g of cyst-material was needed to produce 1 million nauplii; in the worst case, however, it took up to 50 g, signifying a 90% difference in output (Sorgeloos

*et al.*, 1978). As a result commercial aquaculture has been impeded very seriously. This is especially true for the farming of *Macrobrachium* and *Penaeus* which are dependent upon an *Artemia* diet during a long period in their larval development (Bledsoe *et al.*, 1978; Glude, 1978ab; Smith *et al.*, 1978). Third world countries could hardly afford to import the very expensive cysts. At a regional workshop in Indonesia in 1977 it was concluded that "... the inadequate supply of brine shrimp for feeding shrimp larvae remains as the major constraint in the mass propagation of penaeids in Thailand as in the other countries" (ASEAN, 1977).

Although we had announced at the Kyoto 1976 FAO Technical Conference on Aquaculture (Sorgeloos, 1979a) that the cyst shortage was a technical and only a temporal problem, many people remained sceptical. It was not before the end of 1978 that a change in the situation became visible, firstly by the exploitation of several new natural sources of *Artemia* in Europa, Asia, North and South America, and Australia (Sorgeloos, 1979b) and secondly by the successful inoculation of *Artemia* in North-East Brazil (Sorgeloos *et al.*, 1979). According to the latest data available, cyst provisions now exceed 100 metric tons per year.

The increased availability of cysts resulted in competition among dealers and a substantial decrease in prices to about US \$ 35.00 to 40.00 per kilogram (FOB-prices). Thanks to the application of new harvesting techniques, the hatching quality of the cysts put on the market improved and became more reliable. The classic method of harvesting cysts from the shore required an air-classified treatment as a final purification step in order to remove small dirt particles included in the harvest (Helfrich, 1973). During their stay on the shore the cysts are also often subjected to repeated hydration-dehydration cycles which affect the energetic content of the embryos and eventually lead the embryonic development to the breaking stage. In this latter situation many so called "light" cysts are harvested which are in fact empty cyst shells (Sorgeloos *et al.*, 1976; Benijts *et al.*, 1977). A very pure cyst-product which does not need air-classifying processing can be obtained by harvesting the cysts directly from the water surface. Accumulation of cysts on the shore by wind and wave action can be prevented by the construction of dikes or the installation of floating barriers (Sorgeloos, 1978). Since cysts are mainly produced at high salinities, they remain ametabolic even during light rains, provided that the water is sufficiently turbulent to prevent salinity stratification ["... fresh rainfall on a calm lake provides a lower salinity surface layer where eggs could hatch" (Post, 1977)].

### Conditions for maximal hatching output

The production of *Artemia* nauplii by incubation of cysts in seawater is a very simple procedure. However, when working on a larger scale and with high densities of cysts (which is mostly the case in aquaculture enterprises) the use of appropriate techniques is imperative to obtain maximal hatching efficiencies and to minimize the quantity of cysts needed to produce a specific weight or number of *Artemia* nauplii.

During the last years we have had the opportunity to study in detail the effect of various abiotic parameters on the hatching process (review by Sorgeloos, 1979c). Although the quantitative data vary from one geographical strain to another, the qualitative effect of each individual parameter is similar for all strains studied.

## TEMPERATURE

The various effects of water temperature on the hatching metabolism of *Artemia* cysts are summarized in Fig. 1. The fastest hatching rate and the maximal hatching efficiency are attained around 30 °C. It is interesting to note that as long as the cysts have not reached the breaking stage, an increase of the water temperature within the range of about 33 to 40 °C causes a reversible interruption of the cyst metabolism (Sorgeloos, 1975). When hours or even days later, the water temperature is adjusted to the optimal level for hatching, the cyst metabolism is resumed and the nauplii are born. In the meantime, however, the hatching rate decreased as a function of the duration of exposure to the temperatures above the optimum (Sorgeloos *et al.*, 1976 ; Benijts *et al.*, 1977). Molecular biological studies have recently been initiated to study this phenomenon in more detail (Vallejo *et al.*, 1980). In practice, it can be deduced from this observation that cysts which have been exposed by accident (*e.g.* a technical failure of a heating device) for a short period of time to temperatures above 30 °C (but below 40 °C), are not necessarily useless but can be saved by decreasing the temperature of the medium, and can still produce nauplii.

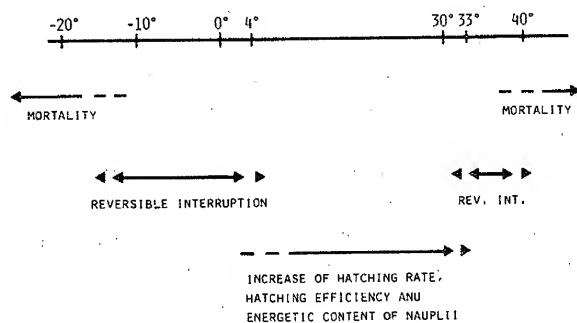


FIG. 1. Schematic diagram of the effect of water temperatures from below -20 °C to over 40 °C, on the cyst metabolism in *Artemia*.

## SALINITY

For reasons of practical convenience natural seawater is mainly used to hatch cysts. Recently, however, it has been demonstrated that at lower salinities the hatching rate increases, that the nauplii have a higher energy content, and that in many cases even higher percentages are scored for the hatching efficiency (Vanhaecke *et al.*, 1980a).

From the "trehalose-glycerol hyperosmotic regulatory system" – theory of Clegg (1964) and Conte *et al.* (1977) we deduced that the increased energy content of the nauplii hatched at 5 ‰ can be explained by the lower levels of glycerol which have to be built up at this salinity to reach the breaking stage (Fig. 2). Since less energy is consumed in hatching, more is left in the nauplius resulting in a higher energy content per unit food available to the predator. In those cases where an increased hatching efficiency was noted, the energetic content of the encysted

embryos might be so close to the critical level needed for hatching that, depending on the level of glycerol that has to be build up, these embryos can reach the breaking stage in water of 5‰ salinity but not in natural seawater.

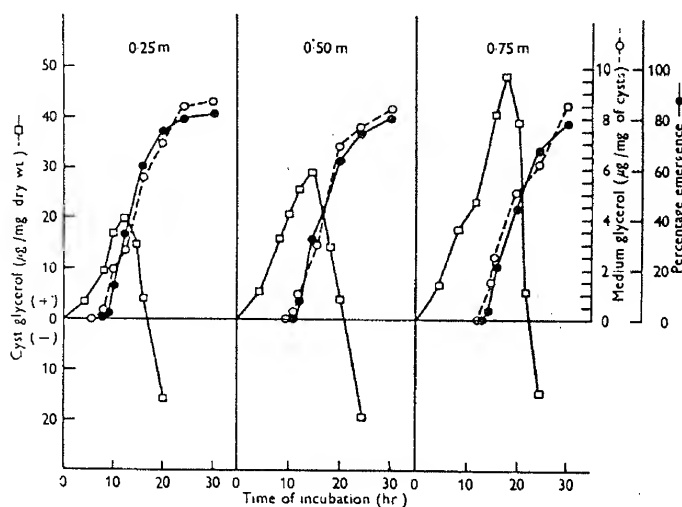


FIG. 2. Relationship between the concentration of glycerol in the cysts (—□—), the glycerol level in the medium (—○—), the percentage of cysts in "breaking" (—●—) and the time of incubation of *Artemia* cysts with 3 different concentrations of NaCl (0.25 m NaCl = 14.6‰ salinity) (after Clegg, 1964).

The 5‰ level has been chosen arbitrarily. At this salinity neither the survival of the hatched nauplii nor their tolerance to salinity stresses is affected. The larvae may indeed be transferred directly and without any harm to seawater of up to 150‰ salinity. The critical minimal salinity for survival has not yet been defined, though it is well-known that in freshwater the physical process of breaking is reached but that the embryos die at the E-1 stage.

## pH

One of the key factors for successful hatching at low salinity is the pH of the medium. Sato (1967) demonstrated that hatching at the E-2 larval stage is triggered by a hatching enzyme that has a maximal activity in the pH-range 8 to 9 (Fig. 3). In diluted seawater media and especially at high cyst densities the buffer capacity of the medium must be increased to keep the pH above 8. This can be achieved by the addition of  $\text{Na}_2\text{CO}_3$  [1 ml of a 0.5 M solution/1 medium (Jones, 1972)] or CaO (65 mg/1 medium). The poor hatching results reported by many authors using artificial seawater or diluted seawater are probably to a large extent related to this pH-effect.

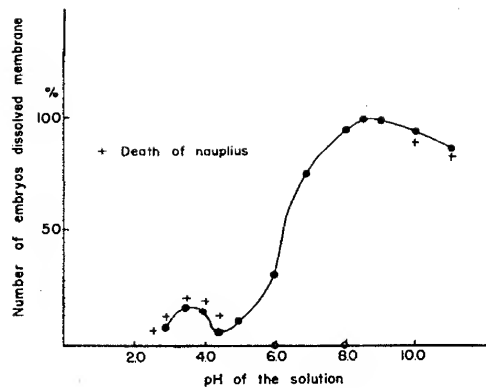


FIG. 3. Influence of the pH of the incubation medium on the activity of the hatching enzyme in *Artemia* embryos (after Sato, 1967).

#### OXYGEN

*Artemia* cysts can be hatched at oxygen concentrations as low as 1 mg/l (Sorgeloos and Persoone, 1975). At lower levels the metabolism is reversibly interrupted. In order to obtain a maximal hatching efficiency, oxygen levels close to saturation are recommended, and most important, all cysts should be kept in suspension. Indeed accumulation of cysts on the bottom of the hatching container creates anaerobic zones which interrupt the cyst's metabolism.

Optimal hatching can be achieved with various types of funnel-shaped containers that are averted from the bottom. We found a very handy solution in using heat-sealed plastic bags made from polyethylene sheets.

#### CYST DENSITY

As demonstrated by Kurata (1967), who incubated up to 17 g cysts/l medium, the hatching process is not affected by cyst density, provided, however, that the other prerequisites are fulfilled. In view of the technical problems encountered in maintaining high oxygen levels without inducing foaming or mechanical injury of the hatched nauplii, it is recommended not to exceed densities of 10 g/l, especially when working with large quantities.

#### ILLUMINATION

When hatching is performed in darkness the hatching success is only half of what it would be if the operation was carried out in light conditions (Sorgeloos, 1973). More recent experiments indicate that not only the hatching efficiency but also the hatching rate are affected by the light intensity (Vanhaecke *et al.*, 1980b). Considering the differences which are observed among strains, a continuous illumination of about 1 000 lux assures a maximal hatching output. This light intensity is attained when the hatching container is placed at about 20 cm from a fluorescent light tube of 60 W.

In order to obtain a maximal energetic output, it does not suffice to incubate the cysts in optimal hatching conditions : one also has to harvest the nauplii at the right moment (Benijts *et al.*, 1976). Upon hatching, the instar I nauplii are not yet able to take up food and completely thrive on their energy reserves. Within a few hours they molt into the 2nd and 3rd larval stage. By this time they have already lost over 20 % of their energy reserves (Fig. 4). Consequently, the most economical use of *Artemia* cysts implies the incubation of the cysts under strictly defined conditions with regard to the abiotic parameters outlined above, and harvest of the larvae in the instar I stage.

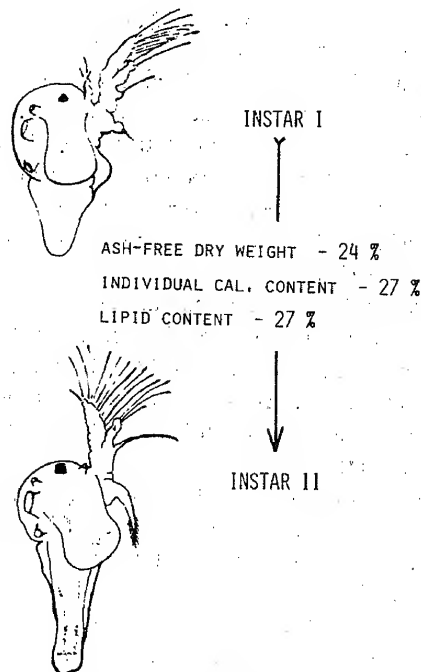


FIG. 4. Changes in energetic content of *Artemia* nauplii upon molting from instar I into instar II-III nauplii.

In spite of the various methods described to facilitate and maximize the separation of nauplii from the hatching debris (Sorgeloos and Persoone, 1975 ; Sorgeloos, 1979a ; Dye, 1980), many limitations still exist. This is particularly true for the separation based on light attraction because nauplii of some strains are negatively phototactic [Venezuela and Spain (Claus *et al.*, 1977)]. In this regard the cyst decapsulation treatment proposed by Sorgeloos *et al.* (1977) with recent improvements by Bruggeman *et al.* (1979, 1980), can be considered as a very important advance in the use of *Artemia* cysts in aquaculture hatcheries. The use of decapsulated cysts indeed not only eliminates all separation problems but has several other advantages, *e.g.* disinfection of the cysts, maximized hatchability, and increased naupliar energetic content. In addition this process opens up the possibility of feeding the cultured species directly on decapsulated cysts (Bruggeman *et al.*, 1980).

### Nutritional value of *Artemia* nauplii as food source in aquaculture hatcheries

With the outlook for increasing *Artemia* cyst provisions better than ever before, the next aspect that deserves urgent attention is the food value of *Artemia* nauplii for various cultured species. Although at the end of 1978 cysts from about a dozen geographical strains of brine shrimp were, or will soon be commercially available<sup>2</sup>, data on the nutritional value of the freshly-hatched nauplii for various cultured species are extremely scarce. We tentatively report the following information :

- Good production results are reported for two freshwater fishes fed nauplii from Burgas-Pomorie, Bulgaria (Lüdsanova and Joshev, 1972), for *Dicentrarchus labrax* fed nauplii from the Çamalti saltern-Izmir, Turkey (Uçal, 1979), for various marine and freshwater fishes fed on several brine shrimp strains from the USSR (Oleynikova and Pleskachevskaya, 1979 ; Spektorova, 1979 and personal communication), for *Palaemon* sp. and *Penaeus japonicus* fed *Artemia* originating from Little Manitou Lake, Canada (respectively Kurata, 1967 ; Fujinaga and Kittaka, 1967), for *Penaeus kerathurus* fed nauplii from San Fernando-Cadiz, Spain (Rodriguez, 1975) and for *Macrobrachium americanum* fed *Artemia* from Manaure, Colombia (Cantillo, 1978).
- No significant differences were reported for the parameters growth and survival of the larvae of *Palaemonetes pugio* fed *Artemia* from Chaplin Lake, Canada or San Francisco Bay, California, USA (Provenzano and Goy, 1976). This Canadian strain, however, performed better than other unspecified *Artemia* strains when offered to *Panulirus interruptus* : "... There is considerable variation in growth and survival of phyllosomes in regards to source of *Artemia*" (Dexter, 1972).
- Fuchs and Person-Le Ruyet (1976) and Person-Le Ruyet and Salaun (1977) did not observe significant production differences when feeding the larvae of *Solea*, *Dicentrarchus*, and *Scophthalmus* with *Artemia* nauplii from either Sète (France), Larnaca Lake (Cyprus) or San Francisco Bay (California, USA).
- Watanabe *et al.* (1978abc, 1979), Watanabe (1979) and Fujita *et al.* (1980) compared *Artemia* nauplii (freshly hatched, starved or fed with enriched diet up to 3 days long) from Canada, San Francisco Bay (California, USA), South America and Tientsin (People's Republic of China) as a food source for red sea bream larvae *Pagrus major*. From their chemical analyses and feeding tests with the red sea bream it appears that high fish mortalities are induced by low levels of the essential fatty acids (EFA) 20:5 $\omega$ 3 and 22:6 $\omega$ 3 in the *Artemia* nauplii. Canadian, Chinese, and San Francisco Bay *Artemia* (two batches of the latter) contained high amounts of EFA, whereas nauplii from South America and from four other San Francisco Bay batches were deficient in EFA.
- High mortalities have been observed for various cultured species fed Great Salt Lake (GSL, Utah, USA) nauplii as sole food source<sup>3</sup> :

<sup>2</sup> Buenos Aires (Argentina), Shark Bay (Australia), Macau-area (Brazil), Chaplin Lake (Canada), Lavalduc (France), Gujarat-area (India), Tientsin (People's Republic of China), Barotac Nuevo (Philippines), Cadiz-area (Spain), San Francisco Bay (California, USA) and Great Salt Lake (Utah, USA).

<sup>3</sup> Here we do not consider the poor performance results with GSL-nauplii that are related to the large size of the nauplius and the resulting inability of the predator to ingest the prey (Smith, 1976).



- . after 3 weeks of feeding on this diet, sole and plaice larvae refused to further ingest these nauplii, did not undergo metamorphosis and died (Shelbourne, 1968); GSL-nauplii were reported by Slobodkin (1968, in Kinne, 1977) to be toxic to plaice larvae;
- . Little (1969), Reed (1969), and Reeve (1969) observed high mortalities in their decapod cultures fed with GSL-*Artemia*;
- . *Palaemon serratus* larvae fed on GSL nauplii died upon metamorphosis (Forster and Wickins, 1967; Wickins, 1972);
- . Bookhout and Costlow (1970) fed Great Salt Lake-nauplii to the larvae of four crab species and reported high mortalities and abnormal developments in the megalopa and first crab stage. Similar observations were published by Roberts (1971, 1974);
- . the total length of the period of larval development in *Palaemonetes pugio* is unaffected by the geographical origin of the brine shrimp diet; much higher mortalities are, however, noted with Great Salt Lake nauplii as compared to San Francisco Bay-*Artemia* (Provenzano and Goy, 1976);
- Matsuoka (1975, in Murai and Andrews, 1978) reported that Chinese *Artemia* nauplii are toxic for the larvae of *Macrobrachium rosenbergii*. Pesticide analyses revealed 5 times and 10 times higher concentrations of DDT, respectively chlorinated hydrocarbons in Chinese *Artemia* compared to SFB-*Artemia*.

Various theories have been advanced to explain the poor performances of the Great Salt Lake *Artemia*-diet:

- residual pesticides from the surrounding agricultural lands are drained into the Great Salt Lake and accumulate in the GSL-*Artemia* (Slobodkin, 1968 in Kinne, 1977);
- through the past centuries GSL-*Artemia* might have developed immunity for a toxic alkaloid secreted by algal blooms in the Great Salt Lake and concentrated in the *Artemia* cysts (Shelbourne, in Provasoli, 1969);
- the Great Salt Lake being of athalassohaline origin, Oppenheimer (in Provasoli, 1969) considered mineral deficiency as the possible source of problems.

The few analytical data that have been published with regard to the chemical composition of GSL-*Artemia* are very confusing:

- Bookhout and Costlow (1970) detected 3 times more DDT in GSL-*Artemia* than in cysts from the San Francisco Bay;
- pesticides, heavy metals, carotenoids, sterols, and fatty acids were analyzed in both SFB and GSL-*Artemia* by Wickins (1972), "... some differences were found but none of them could be confidently labeled as the cause of the poor food value of the Utah *Artemia* nauplii";
- Helfrich and Shigueno (in Helfrich, 1973) found high levels of DDT in both SFB and GSL-nauplii;
- the observation of Wickins (1972) that GSL-nauplii, when fed during 2 days with live algae, become a good food for *Palaemon*-larvae, incited Claus *et al.* (1979) to perform a detailed biochemical analysis of fed and unfed *Artemia* larvae from Great Salt Lake and San Francisco Bay origin. Their results, however, were not conclusive in explaining earlier reports of poor performances of freshly hatched GSL-brine shrimp as a food source in aquaculture hatcheries.

Aside from the specific knowledge with regard to red sea bream culturing in Japan (see above), it is presently impossible to define the chemical and/or nutritional parameters which determine the biological effectiveness of a specific batch or strain of *Artemia* as a good diet for particular cultured species. The analytical methods varied from one study to another, the cyst batches used were never the same, the culturing tests were performed with fish and crustacean larvae which probably show interspecific differences in nutritional requirements and/or sensitiveness.

As a result there is great need for a thorough characterization study of *Artemia* strains which should be undertaken on an interdisciplinary level. Guidelines for the selection of *Artemia* strains for specific uses in the aquaculture hatcheries are urgently needed since at present the choices of new sources of brine shrimp with regard to their potential suitability for the cultured species are arbitrary and as such not without risks.

Such an interdisciplinary research program was initiated in 1978 through the *Artemia* Reference Center at the State University of Ghent in Belgium under the title of "International Study on *Artemia*" (ISA) based on the collaboration of five laboratories from different countries. The participants in this study and their specific research tasks are the following :

- *Artemia* Reference Center, State University of Ghent, Belgium (Coordinator : P. Sorgeloos).  
Biometrical analyses ; hatching, growth, and reproductive characteristics in function of different temperature-salinity combinations ; hybrid tests ; preparation and standardization of research material for the participating laboratories ;
- Department of Food Science and Technology, Nutrition and Dietetics, University of Rhode Island, USA (Coordinator : K. L. Simpson).  
Chemical and biochemical analyses of cysts, nauplii and adults : amino acids, fatty acids, lipids, carotenoids, chlorinated hydrocarbons and heavy metals ;
- Environmental Research Laboratory, Environmental Protection Agency at Narragansett, Rhode Island, USA (Coordinator : A. D. Beck).  
Biological effectiveness of brine shrimp for the fishes *Menidia menidia* and *Pseudopleuronectes americanus*, and the crustaceans *Menippe mercenaria*, *Mysidopsis bahia*, and *Rhithropanopeus harrisii* ; naupliar swimming behavior ;
- Center of Mariculture Research, Port Aransas Marine Laboratory of the University of Texas Marine Science Institute, USA (Coordinator : O. A. Roels).  
Biological effectiveness of brine shrimp for the fish *Cynoscion nebulosus* and the crustacean *Penaeus vanamei* ;
- St. Croix Marine Station, University of Texas Marine Science Institute, US Virgin Islands (Coordinator : O. A. Roels).  
Production performances of *Artemia* in the local Artificial Upwelling Mariculture System ; production of nauplii, cysts and/or adults as testmaterial for the other participating laboratories ;
- Department of Genetics, University College of Swansea, UK (Coordinator : J. A. Beardmore).  
Genotype characterization ; inheritance of specific quantitative characteristics ; temperature and salinity adaptation studies.

Five strains were selected for an initial characterization study :

- Great Salt Lake (Utah, USA) harvest 1977 made available by "Sander's Brine Shrimp Cy" ;
- Macau (Rio Grande do Norte, Brazil) harvest 1978 made available by "Companhia Industrial do Rio Grande do Norte" (CIRNE) ;
- Margherita di Savoia (Italy) harvest 1977 made available by P. Trotta ("Laboratorio per lo Sfruttamento Biologico delle Lagune", Lessina, Italy) ;
- San Francisco Bay (California, USA) batch Living World 1628 purchased from "San Francisco Bay Brand Cy" ;
- Shark Bay (W. Australia) batch 114 made available by "World Ocean Pty".

These strains were selected on the basis of the following criteria : availability ; their use in aquaculture hatcheries (all except Margherita di Savoia) ; same genotype but produced in different habitats [the Macau salt ponds were inoculated with San Francisco Bay *Artemia* in 1977 (Sorgeloos *et al.*, 1979)] ; geographical isolation free from contamination by urban, industrial and/or agricultural waste waters (Macau and Shark Bay) and genetic differences (Margherita di Savoia and Shark Bay are parthenogenetic strains, the others zygogenetic).

During the course of this study we have been informed that Living World batch 1628 cysts were not harvested from San Francisco Bay saltworks (as stated on the label of the commercial product) but from San Pablo Bay salt ponds in the Nappa Valley, north of San Francisco (A. Schmidt, personal communication). Although these two *Artemia* habitats are situated within a few hundred kilometers from each other, the San Pablo Bay drains much more agricultural run-off waters to the ocean than does the San Francisco Bay. In expectation of further data on the exact origin of San Francisco Bay *Artemia sensu lato*, San Pablo Bay *Artemia* ("Living World, San Francisco Bay Brand Cy" batch 1628) are considered as distinct from San Francisco Bay *Artemia* (cysts sold under the label "San Francisco Bay Brand Cy").

The results of the detailed characterization study of the five selected strains of *Artemia* are reported by Johns and Walton (1979), Beck *et al.* (1980), Johns *et al.* (1980), Klein-MacPhee *et al.* (1980), Olney *et al.* (1980), Schauer *et al.* (1980) and Seidel *et al.* (1980). A wider range of strains was studied for their genetic similarities (Abreu-Grobois and Beardmore, 1980), their biometrical characteristics (Vanhaecke and Sorgeloos, 1980a), their growth and production performances on live algae in a batch culturing system (Vanhaecke and Sorgeloos, 1980b) and in a flow-through system (Tobias *et al.*, 1980), their carotenoid composition and metabolism (Soejima *et al.*, 1980), and their naupliar locomotory rates, patterns and photoresponses (Miller *et al.*, 1979).

From this ISA-study it appears that for most parameters studied, considerable variability exists between *Artemia* strains. These initial data already provide pertinent information for the selection and practical use of brine shrimp nauplii in aquaculture : *e.g.* the difference in nutritional value of particular strains for specific predators, the size, biochemical composition, and energetic content of the freshly hatched nauplii, etc. It is clear that this comparative ISA-program should be further extended not only to more cyst samples but also to more test-organisms, in order to further unravel the parameters that define the "suitability" of *Artemia* nauplii as food source in aquaculture hatcheries. In this regard the following *Artemia* strains have been selected for the 1979-1980 ISA-program : *Artemia* Reference Cysts (see Report Workshop I "Characterization of *Artemia* strains for application in aquaculture", this symposium), Chaplin Lake (Canada), Lavalduc (France) and Tientsin (People's Republic of

China). Very valuable research material will furthermore result from the production of *Artemia* cysts in standardized culturing tests with feeding of the brine shrimp with formulated diets containing various amounts of EFA and pesticides.

Through application of the latest developments in quantitative genetics, the ISA-program aims, on a long term basis, to develop new strains of brine shrimp for the benefit of aquaculture: e.g. availability of minute *Artemia* nauplii, smaller than 150  $\mu\text{m}$  in length, could eliminate the need for expensive and cumbersome rotifer production, necessary to culture fishes with very small larvae (milkfish, mullet, turbot, etc.) and crustaceans such as shrimp and crab.

### The use of adult *Artemia* as food source

Although for technical reasons the use of *Artemia* is limited in most cases to freshly hatched nauplii, adult brine shrimp definitely deserve more attention for many reasons:

- adult *Artemia* are 20 times larger and weigh 500 times more than freshly hatched nauplii (Reeve, 1963); their nutritional value changes considerably during growth: the fat content decreases from  $\pm 20\%$  to less than 10% of the dry weight and the protein content increases from  $\pm 42\%$  to over 60% (Von Hentig, 1971; Helfrich, 1973); whereas nauplii are deficient in histidine, methionine, phenylalanine, and threonine, adult brine shrimp are rich in all essential amino acids (Stults, 1974; Gallagher and Brown, 1975; Watanabe *et al.*, 1978a; Claus *et al.*, 1979);
- *Artemia* is a euryhaline and eurythermal crustacean and a non-selective particle filter-feeder; contrary to many other crustaceans its food requirements do not change during growth; it has a high food conversion efficiency and can be cultured in very high densities (Helfrich, 1973; Sorgeloos and Persoone, 1975);
- *Artemia* has a short generation time (minimum of about 2 weeks), a high fecundity rate (up to over 100 offsprings every 4 days) and a long lifespan (up to more than 6 months) (Nimura, 1967; Ivleva, 1969);
- the exoskeleton of the adult is only 1  $\mu\text{m}$  thick which allows consumption of the whole animal without previous processing; for many aquaculture organisms pre-adult or adult *Artemia* are known to be a much better reference diet than formulated feeds: e.g. for *Homarus americanus* (Hughes *et al.*, 1974; Gallagher *et al.*, 1976), *Macrobrachium rosenbergii* (Aquacop, 1977), *Penaeus kerathurus* (San Feliu *et al.*, 1976), *Penaeus aztecus* (Venkatarámiah *et al.*, 1975), *Callinectes sapidus* (Milliken *et al.*, 1980), *Solea solea* and *Scophthalmus maximus* (Aronovich and Spektorova, 1971), *Sparus auratus* (Alessio, 1974) and *Dicentrarchus labrax* (Barahona-Fernandes and Girin, 1976).

In view of its high price (wholesale price up to US \$ 20.00 per kilogram fresh weight), live as well as frozen *Artemia* adults are presently used as a luxury food source in the pet market and, to some extent, for research work in lobster and prawn farming (Anonymous, 1978). Although natural brine shrimp populations are still the most important source of commercially available *Artemia*, they are only exploited in a few areas in Canada, France, and the USA with a total yearly output of approximately 1 000 metric tons. The future output from nature where *Artemia* has to date been recorded from more than 150 habitats (Persoone

and Sorgeloos, 1980) will probably increase considerably. However, new exploitations should be carefully planned, taking into account maximum sustainable yields (in order not to affect cyst production) and the potential local role of *Artemia* as energy source for migrating and breeding waterbirds (Rooth, 1965 ; Herbst and Dana, 1980). New suitable areas for the production of substantial tonnages of *Artemia* biomass (and eventually cysts) can furthermore be considered, without any serious risks for ecological disturbances, by converting thousands of hectares of hypersaline lagoons and abandoned saltponds, which can be found all over the world (Serene and Webber, in Hempel, 1970 ; Rabanal and Beuschel, 1978), into *Artemia*-biotopes ; this implies of course well-defined inoculation and production projects.

Lately, another interesting source of *Artemia* production has come into perspective. Tertiary treatment plants for industrial effluents of high salinity are capable of producing substantial amounts of adult *Artemia*. Attention shall, however, be paid to the eventual bio-accumulation of toxic substances (Milligan *et al.*, 1980).

The present output of brine shrimp from controlled intensive culturing systems is still limited. However, in view of the very important progresses made in this field (see hereunder) the interest in this type of *Artemia* production is increasing considerably. When it comes to a choice, cultured *Artemia* are always to be preferred over brine shrimp harvested from nature. The latter animals indeed often carry parasites or suffer from bacterial and fungal diseases (Persoone and Sorgeloos, 1980) ; furthermore they have mostly been starved for days before being shipped to their final destination.

Since it has been shown that "... progressively larger *Artemia* ... were required by the fish as they grew themselves" (Purdom and Preston, 1977) an adequate and simple technology for cheap production, in the aquaculture hatcheries, of brine shrimp larvae of intermediate size will receive more and more attention (Barahona-Fernandes *et al.*, 1977).

### Progress in controlled intensive *Artemia* culturing

Most of the techniques which have been described in the past for high-density culturing of *Artemia* in batch systems have only limited application. This is due to either the complexity of the technique and/or the limited availability or the high price of the food used (reviews by Bossuyt and Sorgeloos, 1980 ; Dobbeleir *et al.*, 1980).

A major innovation in the technology of *Artemia* batch-culturing with potential for worldwide application is the air-water-lift raceway, originally developed for the intensive culture of post-larval penaeid shrimp (Mock *et al.*, 1973) but modified for brine shrimp culturing at the *Artemia* Reference Center (Sorgeloos *et al.*, 1977). Details on design and construction as well as the description of simple food distribution systems are given by Bossuyt and Sorgeloos (1980). Food dosing in this raceway method is based on readings of water turbidity, which allow automatization of the food distribution by use of turbidimetric devices (Versichele *et al.*, 1979). A cheap and worldwide available food source was found in rice bran (Sorgeloos, 1978 ; Sorgeloos *et al.*, 1980). It now appears that many other types of agricultural waste products, such as whey powder can also be used successfully as a monodiet to culture brine shrimp (Dobbeleir *et al.*, 1980).

Presently 10 g of cysts can be converted into 2 kg pre-adult *Artemia* within 2 weeks in a raceway of 1 m<sup>3</sup>. The protein content and amino acid composition of *Artemia* fed ricebran do

not differ significantly from those of brine shrimp harvested from nature (Sorgeloos *et al.*, 1980). However, in view of the differences in fatty acid composition, further studies are needed to evaluate the nutritional value of brine shrimp raised on waste products for various cultured organisms and, if necessary, to consider diet formulations and/or alterations (Dobbela *et al.*, 1980).

Whereas batch culturing in air-water-lift raceways has a potential worldwide application in aquaculture hatcheries to produce brine shrimp of various sizes, a much more intensified mass production can be achieved in flow-through systems. This is, however, only possible in a very restricted number of situations. In a joint collaboration effort, the St. Croix Marine Station of the University of Texas Marine Science Institute and the *Artemia* Reference Center developed a technique for flow-through culturing of brine shrimp in very high densities (Tobias *et al.*, 1979). The keys to success with this particular technology are the circular screen cylinder (which must be changed regularly) and the cleaning effect of an aeration collar fixed at the bottom of the filter cylinder to keep the screen free from clogging.

The flow-through tests carried out in St. Croix were run with the effluent of the two algal ponds of the local Artificial Upwelling Project (Roels *et al.*, 1976). By extrapolation from repeated 100 l trials (Tobias, personal communication) it has been calculated that 30 g of cysts can be converted in a 1 m<sup>3</sup> tank into 25 kg adult biomass within 2 weeks! The maximal productivity potential of flow-through culturing has not yet been reached in St. Croix. Water temperatures during the period of the experiments were rather low (22-25 °C), and as a consequence of the limited cell densities in the algal cultures (5.10<sup>4</sup> *Chaetoceros curvisetus* cells/ml) the maximum *Artemia* density had to be restricted to 12 000 ind./l. Laboratory tests indicate, however, that densities in flow-through cultures may reach 40 000 *Artemia*/l (Nimura, 1967).

The successes obtained so far in replacing live algae by inert diets such as whey powder or rice bran, will now be studied further. At the same time endeavors will be made to further automatize the flow-through culturing technique.

ISA-studies on the production potential of various *Artemia* races [e.g. differences in growth rate (Tobias *et al.*, 1980; Vanhaecke and Sorgeloos, 1980b); temperature optimum; food conversion efficiency; protein content; *etc.*] indicate that it will become possible to select strains with improved characteristics for intensive culturing.

As pointed out above, the potential sites where *Artemia* flow-through production is possible are much more limited than for batch culturing in closed raceways, especially with regard to the need for large volumes of seawater at a temperature within the range of 20 to 30 °C. As potential sources of alternative energy, Ocean Thermal Energy Conversion systems (OTEC) as well as geothermal projects are gaining more and more interest (Anonymous, 1979; Bardach, 1979). In an OTEC-plant *Artemia* could be grown in the effluent on an inert diet or on phytoplankton cultures produced in an artificial upwelling system of the St. Croix-type (Roels *et al.*, 1976) connected to the same OTEC-plant. Since the salinities of geothermal water range from brackish up to 100 ‰ brine, *Artemia* is the only invertebrate that can be cultured in such a wide salinity range. For the same reason flow-through culturing of brine shrimp can be considered in the warm brine effluent of desalination plants.

Semi-industrial production of *Artemia* should now be started in pilot-plants in order to assess the economical feasibility of a potential annual output of thousands of tons of animal protein from these new bio-industries.

### Potential use of *Artemia* as protein source

From the foregoing it appears that the yearly production of brine shrimp may increase substantially during the next decade. Besides an improved perspective for the use of *Artemia* in the aquaculture hatcheries, it becomes obvious that other applications show very high potential even including direct use in human nutrition. Although the acceptability of brine shrimp as food for man might seem to be speculative or restricted to a few areas in the world, it is certainly worthwhile to be considered, not the least for third world countries. From an energetic point of view brine shrimp production is a much more efficient way to produce animal protein than to culture carnivorous fish and crustaceans with *Artemia* and fish meal as diet ingredients !

Direct consumption of brine shrimp by man has been and is still practised by primitive tribes in America respectively in Africa : "... Indians inhabiting this region used to collect large quantities of this crustacean which they dried and used as food" (Jensen, 1918). The Dawada-people in Libya consume dried *Artemia* flakes as "... a superb source of protein rich in  $\beta$ -carotene and riboflavin" (Ghannudi and Tufail, 1978) and market these "... pains d'*Artemia*" (*Artemia* bread) as a nutritious delicacy over a wide area (Oudney, 1828, in Bovill, 1968 ; Delga *et al.*, 1960 ; Monod, 1969). Taste panel tests on *Artemia* conducted in Hawaii should be further extended since "... the response to an experimental shrimp tempura prepared from frozen brine shrimp was quite favorable" (Davidson, 1974 ; Helfrich, 1973).

If not consumed directly as human food, *Artemia* meal can be used as a rich source of animal protein in livestock diets (Anonymous, 1978). In this regard dried brine shrimp may be used as a valuable alternative to fish meal, especially in those countries that are entirely dependent on fish meal import.

### Concluding remarks

In conclusion it may be said that *Artemia* should no longer be considered as a luxury food in aquaculture but rather as a cheap and high quality source of animal protein. Now more than ever, many aspects dealing with the use of *Artemia* in aquaculture need to be studied further in order to reach this goal. A first promising step in this direction is the Aquaculture Planning Program for Hawaii (Anonymous, 1978) in which brine shrimp has been considered as a first priority species.

In his mathematical evaluation of the overall value and market potential of 24 of the most important aquaculture candidate species or groups of species, Nash (1974, in Kinne and Rosenthal, 1977) ranks brine shrimp second after salmon (Table I). The recent finding of cheap inert diets for brine shrimp lifts this species to the top place in rank of importance in the field of aquaculture.

TABLE I  
Overall state of the art and market potential  
of 24 of the most important aquaculture candidate species or groups of species  
[1 : no : 5 : yes : 2 to 4 : in-between scorings (after Nash, 1974, in Kinne and Rosenthal, 1977)]

Parameter	Score																							
Controlled spawning possible	5	5	5	4	4	2	1	3	1	5	5	4	4	1	1	1	2	4	1	3	1	2	4	1
Simple larval development achieved	5	5	5	5	5	4	5	5	2	5	5	5	2	1	1	1	3	5	3	3	5	3	4	1
Mass-produced in hatchery	5	5	5	4	3	1	4	4	1	5	5	5	1	1	1	1	1	1	2	1	1	1	4	1
Fast growth rate potential	5	5	4	4	4	3	4	4	4	5	5	4	4	4	5	5	5	4	4	5	5	4	3	3
Satisfactory feeds known	5	4	4	3	3	1	3	3	3	5	5	3	3	1	2	1	5	3	3	5	5	3	3	2
Commercial feeds available	1	1	1	1	1	1	2	1	1	1	5	1	1	1	1	1	3	1	1	2	1	1	1	1
High conversion efficiency	2	2	2	2	2	3	3	3	3	4	5	4	3	2	4	4	4	3	3	5	5	3	2	1
Hardy in captivity	5	5	3	3	3	5	3	3	3	5	5	5	5	3	3	3	5	2	3	5	5	2	3	3
High disease resistance	4	4	4	4	4	4	3	4	3	5	4	4	4	3	3	3	4	2	3	4	4	3	2	4
High density potential	5	5	5	5	4	5	3	3	5	5	4	4	4	3	4	4	4	5	5	5	4	3	3	5
Farming systems developed	5	5	3	3	3	1	4	2	1	5	4	4	4	1	1	3	5	1	1	5	5	1	3	4
High price range	5	2	4	4	4	1	4	5	1	5	5	3	2	1	4	4	5	2	2	5	1	4	5	3
High market potential U.S.	5	1	5	5	5	2	5	5	1	5	5	3	1	1	5	5	4	3	3	2	1	3	5	5
High market potential foreign	5	5	5	5	5	3	5	5	3	5	5	4	4	5	4	5	4	4	4	5	5	5	5	3
Matrix total	62	54	55	52	51	36	49	50	32	65	67	53	42	27	40	55	40	37	56	48	38	47	37	
	Oysters	Mussels	Clams	Scallops	Abalone	Crabs	Shrimps	Lobster	Krill	Artemia	Salmon	Flatfish	Mullet	Rabbitfish	Dolphinfish	Pompano	Yellow-tail	Anchovy	Herrings	Eels	Milkfish	Octopus	Turtles	Bloodworm

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